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(54) Title: hB7-H2, A NOVEL CO-STIMULATORY MOLECULE

(57) Abstract: The invention provides novel hB7-H2 polypeptides useful for co-stimulating T cells, isolated nucleic acid molecules encoding them, vectors containing the nucleic acid molecules, and cells containing the vectors. Also included are methods of making and using these co-stimulatory polypeptides.

hB7-H2, A NOVEL CO-STIMULATORY MOLECULE

BACKGROUND OF THE INVENTION

The invention is generally in the field of immunoregulation, and specifically T
5 cell response regulation.

Mammalian T lymphocytes recognize antigenic peptides bound to major
histocompatibility complex (MHC) molecules on the surface of antigen presenting
cells (APC). The antigenic peptides are generated by proteolytic degradation of
protein antigens within the APC. The interaction of the T cells with the APC and the
10 subsequent responses of the T cells are qualitatively and quantitatively regulated by
interactions between cell surface receptors on the T cells with both soluble mediators
and ligands on the surface of APC.

SUMMARY OF THE INVENTION

15 The invention is based on the cloning of a human cDNA molecule containing
a sequence encoding a novel molecule that co-stimulates T cell responses and the
functional characterization of the polypeptide that the cDNA molecule encodes. The
polypeptide is designated hB7-H2. The invention features DNA molecules encoding
the hB7-H2 polypeptide, functional fragments of the polypeptide, and fusion proteins
20 containing the polypeptide or functional fragments of it, hB7-H2 and functional
fragments of it, fusion proteins containing the polypeptide or functional fragments
vectors containing the DNA molecules, and cells containing the vectors. Also
included in the invention are antibodies that bind to the hB7-H2 polypeptide. The
invention features *in vitro*, *in vivo*, and *ex vivo* methods of co-stimulating T cell
25 responses, methods of screening for compounds that inhibit or enhance T cell
responses, and methods for producing the above polypeptides and fusion proteins.

Specifically, the invention features an isolated DNA including: (a) a nucleic
acid sequence that encodes a hB7-H2 polypeptide with the ability to co-stimulate a T
cell. The encoded polypeptide is less than 555 (*i.e.*, less than 540, 520, 500, 480, 460,

44, 420, 410, 390, 370, 350, 330, or 310) amino acids in length and the nucleic acid sequence hybridizes under highly stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence with SEQ ID NO:1. Alternatively, the isolated DNA can include the complement of the above nucleic acid sequence. In the DNA of the invention, if the polypeptide, when its amino acid sequence is aligned with SEQ ID NO:1, includes a first amino acid residue at a position equivalent to position 301 of wild-type polypeptide (SEQ ID NO:1) the first amino acid residue is histidine or a conservative substitution thereof. If the polypeptide, when its amino acid sequence is aligned with SEQ ID NO:1, includes a second amino acid residue at a position equivalent to position 302 of wild-type polypeptide (SEQ ID NO:1) the second amino acid residue is valine or a conservative substitution thereof. The nucleic acid sequence included in the isolated DNA will be at least 10 bp, 15 bp, 25 bp, 50 bp, 75 bp, 100 bp, 125 bp, 150 bp, 175 bp, 200 bp, 250 bp, 300 bp, 350 bp, 400 bp, 450 bp, 500 bp, 550 bp, 600 bp, 650 bp, 700 bp, 750 bp, 800 bp, 850 bp, or 900 bp long. The nucleic acid sequence can encode a hB7-H2 polypeptide that includes an amino sequence with SEQ ID NO:1 or it can have a nucleotide sequence with SEQ ID NO:2. The nucleic acid sequence can also encode functional fragments of these hB7-H2 polypeptides.

The invention also embodies isolated hB7-H2 polypeptides encoded by any of the above DNAs. The hB7-H2 polypeptide can include an amino sequence of amino acid residue 22 to amino acid residue 302 of SEQ ID NO:1. The invention also encompasses hB7-H2 polypeptides that include an amino acid sequence with SEQ ID NO:1, or this amino acid sequence but differing solely by one or more conservative substitutions. The polypeptides of the invention include fusion proteins containing a first domain and at least one additional domain. The first domain can be any of the hB7-H2 polypeptides described above or a functional fragment of any of these polypeptides. The at least one additional domain can be a heterologous targeting or leader sequence, an amino acid sequence that facilitates purification, detection, or solubility of the fusion protein. The second domain can be, for example, all or part of an immunoglobulin (Ig) heavy chain constant region. Also included are isolated DNAs encoding the fusion proteins.

The invention features vectors containing any of the DNAs of the invention and nucleic acid molecules encoding the fusion proteins of the invention. The vectors can be expression vectors in which the nucleic acid coding sequence or molecule is operably linked to a regulatory element which allows expression of the nucleic acid sequence or molecule in a cell. Also included in the invention are cells (e.g., mammalian, insect, yeast, fungal, or bacterial cells) containing any of the vectors of the invention.

Another embodiment of the invention is a method of co-stimulating a T cell that involves contacting the T cell with any of the hB7-H2 polypeptides of the invention, functional fragments thereof, or fusion proteins of the invention; these classes of molecule are, for convenience, designated "hB7-H2 agents". The contacting can be by culturing any of these hB7-H2 agents with the T cell *in vitro*. Alternatively, the T cell can be in a mammal and the contacting can be, for example, by administering any of the hB7-H2 agents to the mammal or administering a nucleic acid encoding the hB7-H2 agent to the mammal. In addition, the method can be an *ex vivo* procedure that involves providing a recombinant cell which is the progeny of a cell obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding any of the hB7-H2 agents so that the cell expresses the hB7-H2 agent; and administering the cell to the mammal. In this *ex vivo* procedure, the cell can be an antigen presenting cell (APC) that expresses the hB7-H2 agent on its surface. Furthermore, prior to administering to the mammal, the APC can be pulsed with an antigen or an antigenic peptide. In any of the above methods, the mammal can be suspected of having, for example, an immunodeficiency disease, an inflammatory condition, or an autoimmune disease.

The invention includes a method of identifying a compound that inhibits an immune response. The method involves: providing a test compound; culturing, together, the compound, one or more hB7-H2 agents, a T cell, and a T cell activating stimulus; and determining whether the test compound inhibits the response of the T cell to the stimulus, as an indication that the test compound inhibits an immune response. The invention also embodies a method of identifying a compound that enhances an immune response. The method involves: providing a test compound;

culturing, together, the compound, one or more of hB7-H2 agents, a T cell, and a T cell activating stimulus; and determining whether the test compound enhances the response of the T cell to the stimulus, as an indication that the test compound enhances an immune response. In both these methods, the stimulus can be, for
5 example, an antibody that binds to a T cell receptor or a CD3 polypeptide. Alternatively, the stimulus can be an alloantigen or an antigenic peptide bound to a major histocompatibility complex (MHC) molecule on the surface of an antigen presenting cell (APC). The APC can be transfected or transformed with a nucleic acid encoding the hB7-H2 agent and the hB7-H2 agent can be expressed on the
10 surface of the APC.

The invention also features an antibody (e.g., a polyclonal or a monoclonal antibody) that binds to any of the hB7-H2 polypeptides of the invention, e.g., the polypeptide with SEQ ID NO:1.

The invention also features a method of producing any of the hB7-H2
15 polypeptides of the invention, functional fragments thereof, or fusion proteins of the invention. The method involves culturing a cell of the invention and purifying the relevant hB7-H2 protein from the culture.

"Polypeptide" and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or post-translational modification.
20 The invention also features hB7-H2 polypeptides with conservative substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine.

25 The term "isolated" polypeptide or peptide fragment as used herein refers to a polypeptide or a peptide fragment which either has no naturally-occurring counterpart (e.g., a peptidomimetic), or has been separated or purified from components which naturally accompany it, e.g., in tissues such as pancreas, liver, spleen, ovary, testis, muscle, joint tissue, neural tissue, gastrointestinal tissue, or body fluids such as blood,
30 serum, or urine. Typically, the polypeptide or peptide fragment is considered "isolated" when it is at least 70%, by dry weight, free from the proteins and naturally-

occurring organic molecules with which it is naturally associated. Preferably, a preparation of a polypeptide (or peptide fragment thereof) of the invention is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, the polypeptide (or the peptide fragment thereof), respectively, of the invention.

5 Thus, for example, a preparation of polypeptide x is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, polypeptide x. Since a polypeptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, the synthetic polypeptide or nucleic acid is "isolated."

10 An isolated polypeptide (or peptide fragment) of the invention can be obtained, for example, by extraction from a natural source (e.g., from human tissues or bodily fluids); by expression of a recombinant nucleic acid encoding the peptide; or by chemical synthesis. A peptide that is produced in a cellular system different from the source from which it naturally originates is "isolated," because it will be separated
15 from components which naturally accompany it. The extent of isolation or purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

An "isolated DNA" means DNA free of the genes that flank the gene of interest in the genome of the organism in which the gene of interest naturally occurs.

20 The term therefore includes a recombinant DNA incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote. It also includes a separate molecule such as: a cDNA where the corresponding genomic DNA has introns and therefore a different sequence; a genomic fragment; a fragment produced by polymerase chain reaction (PCR); a
25 restriction fragment; a DNA encoding a non-naturally occurring protein, fusion protein, or fragment of a given protein; or a nucleic acid which is a degenerate variant of a naturally occurring nucleic acid. In addition, it includes a recombinant nucleotide sequence that is part of a hybrid gene, *i.e.*, a gene encoding a fusion protein. Also included is a recombinant DNA that includes a portion of SEQ ID NO:2.

30 As used herein, a polypeptide that "co-stimulates" a T cell is a polypeptide that, upon interaction with a cell-surface molecule on the T cell, enhances the

response of the T cell. The T cell response that results from the interaction will be greater than the response in the absence of the polypeptide. The response of the T cell in the absence of the co-stimulatory polypeptide can be no response or it can be a response significantly lower than in the presence of the co-stimulatory polypeptide. It is understood that the response of the T cell can be an effector, helper, or suppressive response.

As used herein, an "activating stimulus" is a molecule or molecular complex that delivers an activating signal to a T cell, preferably through the antigen specific T cell receptor (TCR). The activating stimulus can be sufficient to elicit a detectable response in the T cell. Alternatively, the T cell may require co-stimulation (e.g., by a hB7-H2 polypeptide) in order to respond detectably to the activating stimulus. Examples of activating stimuli include, without limitation, antibodies that bind to the TCR or to a polypeptide of the CD3 complex that is physically associated with the TCR on the T cell surface, alloantigens, antigenic peptides bound to MHC molecules, or lectin molecules (e.g., concanavalin A (ConA) or phytohemagglutinin (PHA)).

As used herein, a "fragment" of a hB7-H2 polypeptide is a fragment of the polypeptide that is shorter than the full-length polypeptide. Generally, fragments will be five or more amino acids in length. An antigenic fragment has the ability to be recognized and bound by an antibody.

As used herein, a "functional fragment" of an hB7-H2 polypeptide is a fragment of the polypeptide that is shorter than the full-length polypeptide and has the ability to co-stimulate a T cell. Methods of establishing whether a fragment of an hB7-H2 molecule is functional are known in the art. For example, fragments of interest can be made by either recombinant, synthetic, or proteolytic digestive methods. Such fragments can then be isolated and tested for their ability to co-stimulate T cells by procedures described herein.

As used herein, "operably linked" means incorporated into a genetic construct so that expression control sequences effectively control expression of a coding sequence of interest.

As used herein, the term "antibody" refers not only to whole antibody molecules, but also to antigen-binding fragments, e.g., Fab, F(ab')₂, Fv, and single chain Fv fragments. Also included are chimeric antibodies.

As used herein, an antibody that "binds specifically" to an isolated hB7-H2 polypeptide encoded by a DNA that includes a nucleic acid sequence that: (i) encodes a polypeptide with the ability to co-stimulate a T cell; (ii) comprises the last two codons of SEQ ID NO:2; and (iii) hybridizes under stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence with SEQ ID NO:1, is an antibody that does not bind to B7-1, B7-2, or B7-H1 polypeptides. B7-H1 is described in pending application no. 09/451,291.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

Other features and advantages of the invention, e.g., enhancing immune responses in mammalian subjects, will be apparent from the following description, from the drawings and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a depiction of the amino acid sequence of hB7-H2 (SEQ ID NO:1).

FIG. 1B is a depiction of the amino acid sequences of the extracellular domains of hB7-H2, hB7-H1, B7-1, and B7-2 aligned for maximum homology. Identical residues are shaded and conserved residues are boxed.

FIG. 2A is a depiction of the nucleotide sequence (SEQ ID NO:3) of part of a cDNA fragment generated by a PCR protocol using primers with nucleotide

sequences derived from an expressed sequence tag (EST) with Accession No. KIAA0653 and a human dendritic cell (DC) cDNA library as a source of template. SEQ ID NO:3 includes the coding sequence (SEQ ID NO:2) for hB7-H2.

FIG. 2B is a depiction of the nucleotide sequence (SEQ ID NO:2) of cDNA
5 encoding hB7-H2.

FIG. 3 is a photograph of a Northern blot showing expression of hB7-H2 mRNA in various human tissues.

FIG. 4 is a series of fluorescence flow cytometry histograms showing the expression of B7-2 (left) and hB7-H2 (right) on unactivated (top) and
10 lipopolysaccharide (LPS) activated (bottom) DC. The unshaded profiles were obtained with cells stained with antibodies specific for either B7-2 or hB7-H2 and the shaded profiles were obtained with cells stained with control immunoglobulin (Ig).

FIG. 5A is a series of fluorescence flow cytometry histograms showing the binding of hB7-H2hIg to T cells after 0-72 hours of activation with
15 phytohemagglutinin (PHA). The unshaded profiles were obtained with cells stained with hB7-H2hIg and the shaded profiles were obtained with cells stained with control Ig.

FIG. 5B is a series of fluorescence flow cytometry histograms showing the inhibition of binding of hB7-H2hIg to PHA activated T cells by ICOShIg but not by
20 CTLA4hIg. The unshaded profiles were obtained with cells stained with hB7-H2hIg and the shaded profiles were obtained with cells stained with control Ig.

FIG. 5C is a pair of fluorescence flow cytometry histograms showing the binding of ICOShIg (bottom) and antibodies specific for hB7-H2 (top) to 293 cells transfected with and expressing cDNA encoding full-length hB7-H2 (solid line) or a
25 control vector (dotted line).

FIG. 6A is a line graph showing the ability of hB7-H2hIg (open circles) or control Ig (closed circles) (each bound at a concentration of 5 μ g/ml to plastic tissue culture wells) to co-stimulate the proliferation of human T cells in response to
30 activation by mAb specific for human CD3 bound at various concentrations to the plastic tissue culture wells.

FIG. 6B is a line graph showing the ability of hB7-H2hIg (open circles) or control Ig (closed circles) (each bound at various concentrations to plastic tissue culture wells) to co-stimulate the proliferation of human T cells in response to activation by monoclonal antibody (mAb) specific for human CD3 bound at 20 ng/ml to the plastic tissue culture wells.

FIG. 7A is a pair of bar graphs showing the ability of hB7-H2hIg, hB7-H1hIg, control Ig (each bound at a concentration of 5 μ g/ml to plastic tissue culture wells), and soluble mAb specific for human CD28 (at a concentration of 5 μ g/ml) to co-stimulate the production of interleukin (IL)-2 (top graph) and IL-10 (bottom graph) by human T cells activated by antibody specific for human CD3 bound at a high concentration (500 ng/ml) to the plastic tissue culture wells.

FIG. 7B is a pair of bar graphs showing the ability of hB7-H2hIg, hB7-H1hIg, control Ig (each bound at a concentration of 5 μ g/ml to plastic tissue culture wells), and soluble mAb specific for human CD28 (at a concentration of 5 μ g/ml) to co-stimulate the production of interleukin (IL)-2 (top graph) and IL-10 (bottom graph) by human T cells activated by antibody specific for human CD3 bound at a low concentration (40 ng/ml) to the plastic tissue culture wells.

DETAILED DESCRIPTION

Using PCR primers with sequences derived from an expressed sequence tag (EST) that had significant homology to human B7-1 and human B7-2 and a human cDNA library as a source of template, a PCR product that included an open reading frame (orf) encoding hB7-H2 dendritic cell (DC) was generated, isolated, and sequenced. In FIG. 1 is shown the nucleotide sequence (SEQ ID NO:3) of most of this PCR product. The sequence of the coding region (nucleotides 51-956 of SEQ ID NO:3) is designated SEQ ID NO:2.

Translation of the coding region indicated that the polypeptide (SEQ ID NO:1) it encodes (hB7-H2) is a type I transmembrane protein of 302 amino acids containing a signal peptide, an immunoglobulin (Ig) V-like domain, Ig C-like domain, a transmembrane domain and a cytoplasmic domain. Northern blot analysis showed

abundant expression of the gene encoding hB7-H2 in kidney, brain, and peripheral blood mononuclear cells (PBMC), and significant expression in thymus, spleen, heart, and skeletal muscle.

5 An antiserum specific for the extracellular region of hB7-H2 was produced by immunization of mice with a recombinantly produced fusion protein (hB7-H2mIg) that included the extracellular region of hB7-H2 and part of the constant region of mouse IgG2a heavy chain. Fluorescence flow cytometry analysis with this antiserum indicated negligible surface expression of hB7-H2 on unfractionated PBMC. On the other hand, DC generated by culturing adherent PBMC with granulocyte/macrophage-
10 colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) expressed significant levels of cell-surface hB7-H2. However, further maturation of DC lead to a decrease in surface expression of hB7-H2, but not B7-2. Thus, hB7-H2 is preferentially expressed on immature DC.

The failure of a fusion protein (hB7-H2hIg) containing the extracellular region
15 of hB7-H2 fused to part of the constant region of human IgG1 heavy chain to bind to resting PBMC indicated that the counter-receptor for hB7-H2 is not expressed on resting T cells, and thus is not CD28. However, hB7-H2hIg did bind to T cells activated by either PHA or a mixture of antibodies specific for CD3 and CD28. Thus, expression of the counter-receptor for hB7-H2 is inducible on T cells. Binding of
20 hB7-H2hIg to PHA activated T cells was inhibited by a fusion protein containing the extracellular region of ICOS fused to part of the constant region of human IgG1 heavy chain (ICOShIg) but was not inhibited by an analogous fusion protein containing the extracellular region of CTLA4 (CTLA4hIg). These data indicate that ICOS (but not CTLA4) is the counter-receptor on activated T cells for hB7-H2. This contention was
25 confirmed by the observation that ICOShIg bound to 293 cells transfected with and expressing on their surface the coding sequence of hB7-H2 but did not bind to mock transfected 293 cells or 293 cells transfected with and expressing the coding sequence for B7-1.

In vitro experiments with isolated human T cells indicated that hB7-H2hIg
30 enhanced ("co-stimulated") T cell proliferative responses induced by a suboptimal dose of antibody specific for human CD3. In addition, hB7-H2Ig co-stimulated the

production of high levels of interleukin-10 (IL-10) but not interleukin-2 (IL-2) in the presence of a dose of antibody specific for CD3 that was optimal for the induction of T cell proliferation. On the other hand, in the presence of a suboptimal amount of anti-CD3 antibody, hB7-H2 co-stimulated the production of relatively high levels of IL-2 but only a low level of IL-10. Thus, the pattern of cytokines produced in response to hB7-H2 co-stimulation depends on the "strength" of the T cell stimulus.

hB7-H2 can be useful as an augmenter of immune responses both *in vivo* and *in vitro*. Furthermore, in light of its ability to selectively enhance IL-10 production in the context of a potent T cell stimulus, hB7-H2 can be useful in controlling pathologic cell-mediated conditions (e.g., those induced by infectious agents such *Mycobacterium tuberculosis* or *M. leprae*) or other pathologic cell-mediated responses such as those involved in autoimmune diseases (e.g., rheumatoid arthritis (RA), multiple sclerosis (MS), or insulin-dependent diabetes mellitus (IDDM)).

15 hB7-H2 Nucleic Acid Molecules

The hB7-H2 nucleic acid molecules of the invention can be cDNA, genomic DNA, synthetic DNA, or RNA, and can be double-stranded or single-stranded (*i.e.*, either a sense or an antisense strand). Segments of these molecules are also considered within the scope of the invention, and can be produced by, for example, the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription. Preferably, the nucleic acid molecules encode polypeptides that, regardless of length, are soluble under normal physiological conditions. Naturally, the membrane forms are not soluble.

25 The nucleic acid molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide (for example, the polypeptides with SEQ ID NO:1). In addition, these nucleic acid molecules are not limited to coding sequences, e.g., they can include some or all of the non-coding

sequences that lie upstream or downstream from a coding sequence. The nucleic acid molecules can, for example, include SEQ ID NO:3.

The nucleic acid molecules of the invention can be synthesized (for example, by phosphoramidite-based synthesis) or obtained from a biological cell, such as a cell
5 of a mammal. Thus, the nucleic acids can be those of a human, non-human primate (e.g., monkey) mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, dog, or cat.

In addition, the isolated nucleic acid molecules of the invention encompass segments that are not found as such in the natural state. Thus, the invention encompasses recombinant nucleic acid molecules, (for example, isolated nucleic acid
10 molecules encoding hB7-H2) incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefor are discussed further below.

Certain nucleic acid molecules of the invention are antisense molecules or are
15 transcribed into antisense molecules. These can be used, for example, to down-regulate translation of hB7-H2 mRNA within a cell.

Techniques associated with detection or regulation of genes are well known to skilled artisans and such techniques can be used to diagnose and/or treat disorders associated with aberrant hB7-H2 expression. Nucleic acid molecules of the invention
20 are discussed further below in the context of their therapeutic utility.

A hB7-H2 family gene or protein can be identified based on its similarity to the relevant hB7-H2 gene or protein, respectively. For example, the identification can be based on sequence identity. The invention features isolated nucleic acid molecules which are at least 50% (or 55%, 65%, 75%, 85%, 95%, or 98%) identical to: (a) a
25 nucleic acid molecule that encodes the polypeptide of SEQ ID NO:1 (b) the nucleotide sequence of SEQ ID NO:2; or (c) a nucleic acid molecule which includes a segment of at least 30 (e.g., at least 50, 60, 100, 125, 150, 175, 200, 250, 300, 325, 350, 375, 400, 425, 450, 500, 550, 600, 650, 700, 850, or 900) nucleotides of SEQ ID NO:2.

The determination of percent identity between two sequences is accomplished using the mathematical algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* 90, 5873-5877, 1993. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) *J. Mol. Biol.* 215, 403-410. BLAST nucleotide searches are performed with the BLASTN program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to hB7-H2-encoding nucleic acids. BLAST protein searches are performed with the BLASTP program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to hB7-H2. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) *Nucleic Acids Res.* 25, 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used (See <http://www.ncbi.nlm.nih.gov>).

Hybridization can also be used as a measure of homology between two nucleic acid sequences. A hB7-H2-encoding nucleic acid sequence, or a portion thereof, can be used as a hybridization probe according to standard hybridization techniques. The hybridization of a hB7-H2 probe to DNA from a test source (e.g., a mammalian cell) is an indication of the presence of hB7-H2 DNA in the test source. Hybridization conditions are known to those skilled in the art and can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y., 6.3.1-6.3.6, 1991. Moderate hybridization conditions are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by one or more washes in 1 X SSC, 0.1% SDS at 50-60°C. Highly stringent conditions are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by one or more washes in 0.2 X SSC, 0.1% SDS at 50-65°C.

The invention also encompasses: (a) vectors that contain any of the foregoing hB7-H2-related coding sequences and/or their complements (that is, "antisense" sequence); (b) expression vectors that contain any of the foregoing hB7-H2-related coding sequences operatively associated with any transcriptional/translational regulatory elements (examples of which are given below) necessary to direct expression of the coding sequences; (c) expression vectors containing, in addition to sequences encoding a hB7-H2 polypeptide, nucleic acid sequences that are unrelated

to nucleic acid sequences encoding hB7-H2, such as molecules encoding a reporter, marker, or a signal peptide, e.g., fused to hB7-H2; and (d) genetically engineered host cells that contain any of the foregoing expression vectors and thereby express the nucleic acid molecules of the invention.

5 Recombinant nucleic acid molecules can contain a sequence encoding hB7-H2 having an heterologous signal sequence. The full length hB7-H2 polypeptide, a domain of hB7-H2, or a fragment thereof may be fused to additional polypeptides, as described below. Similarly, the nucleic acid molecules of the invention can encode the mature form of hB7-H2 or a form that includes an exogenous polypeptide which
10 facilitates secretion.

 The transcriptional/translational regulatory elements referred to above and which are further described below, include, but are not limited to, inducible and non-inducible promoters, enhancers, operators and other elements, which are known to those skilled in the art, and which drive or otherwise regulate gene expression. Such
15 regulatory elements include but are not limited to the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the
20 yeast α -mating factors.

 Similarly, the nucleic acid can form part of a hybrid gene encoding additional polypeptide sequences, for example, sequences that function as a marker or reporter. Examples of marker or reporter genes include β -lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside
25 phosphotransferase (neo^r, G418^r), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β -galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, additional sequences that can
30 serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being a hB7-H2

polypeptide and the second portion being, for example, the reporter described above or an Ig constant region or part of an Ig constant region, e.g., the CH2 and CH3 domains of IgG2a heavy chain.

The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (for example, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules of the invention; yeast (for example, *Saccharomyces* and *Pichia*) transformed with recombinant yeast expression vectors containing the nucleic acid molecules of the invention (preferably containing the nucleic acid sequence encoding hB7-H2 (e.g., that contained within SEQ ID NO:1)); insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules of the invention; plant cell systems infected with recombinant virus expression vectors (for example, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti plasmid) containing hB7-H2 nucleotide sequences; or mammalian cell systems (for example, COS, CHO, BHK, 293, VERO, HeLa, MDCK, WI38, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (for example, the metallothionein promoter) or from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter). Also useful as host cells are primary or secondary cells obtained directly from a mammal, transfected with a plasmid vector or infected with a viral vector.

Polypeptides and Polypeptide Fragments

The polypeptides of the invention include hB7-H2 and functional fragments of hB7-H2. The polypeptides embraced by the invention also include fusion proteins which contain either full-length hB7-H2 or a functional fragment of it fused to unrelated amino acid sequence. The unrelated sequences can be additional functional domains or signal peptides. Signal peptides are described in greater detail and exemplified below.

The polypeptides can be purified from natural sources (e.g., blood, serum plasma, tissues or cells such as T cells or any cell that naturally produces hB7-H2). Smaller peptides (less than 50 amino acids long) can also be conveniently synthesized by standard chemical means. In addition, both polypeptides and peptides can be produced by standard *in vitro* recombinant DNA techniques and *in vivo* recombination/genetic recombination (e.g., transgenesis), using the nucleotide sequences encoding the appropriate polypeptides or peptides. Methods well known to those skilled in the art can be used to construct expression vectors containing relevant coding sequences and appropriate transcriptional/translational control signals. See, for example, the techniques described in Sambrook et al., Molecular Cloning: A Laboratory Manual (2nd Ed.) [Cold Spring Harbor Laboratory, N.Y., 1989], and Ausubel et al., Current Protocols in Molecular Biology, [Green Publishing Associates and Wiley Interscience, N.Y., 1989].

Polypeptides and fragments of the invention also include those described above, but modified for *in vivo* use by the addition, at the amino- and/or carboxyl-terminal ends, of a blocking agent to facilitate survival of the relevant polypeptide *in vivo*. This can be useful in those situations in which the peptide termini tend to be degraded by proteases prior to cellular uptake. Such blocking agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the peptide to be administered. This can be done either chemically during the synthesis of the peptide or by recombinant DNA technology by methods familiar to artisans of average skill.

Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Likewise, the peptides can be covalently or noncovalently coupled to pharmaceutically acceptable "carrier" proteins prior to administration.

Also of interest are peptidomimetic compounds that are designed based upon the amino acid sequences of the functional peptide fragments. Peptidomimetic compounds are synthetic compounds having a three-dimensional conformation (*i.e.*, a

"peptide motif") that is substantially the same as the three-dimensional conformation of a selected peptide. The peptide motif provides the peptidomimetic compound with the ability to co-stimulate T cells in a manner qualitatively identical to that of the hB7-H2 functional peptide fragment from which the peptidomimetic was derived.

- 5 Peptidomimetic compounds can have additional characteristics that enhance their therapeutic utility, such as increased cell permeability and prolonged biological half-life.

The peptidomimetics typically have a backbone that is partially or completely non-peptide, but with side groups that are identical to the side groups of the amino
10 acid residues that occur in the peptide on which the peptidomimetic is based. Several types of chemical bonds, e.g., ester, thioester, thioamide, retroamide, reduced carbonyl, dimethylene and ketomethylene bonds, are known in the art to be generally useful substitutes for peptide bonds in the construction of protease-resistant peptidomimetics.

15

Methods of Therapy

- The methods of the invention involve contacting a T cell with a hB7-H2 molecule of the invention, or a functional fragment thereof, in order to co-stimulate the T cell. The contacting can occur before, during, or after activation of the T cell.
- 20 Contacting of the T cell with the hB7-H2 polypeptide will preferably be at substantially the same time as activation. Activation can be, for example, by exposing the T cell to an antibody that binds to the TCR or one of the polypeptides of the CD3 complex that is physically associated with the TCR. Alternatively, the T cell can be exposed to either an alloantigen (e.g., a MHC alloantigen) on, for example, an antigen
25 presenting cell (APC) (e.g., a dendritic cell, a macrophage, a monocyte, or a B cell) or an antigenic peptide produced by processing of a protein antigen by any of the above APC and presented to the T cell by MHC molecules on the surface of the APC. The T cell can be a CD4+ T cell or a CD8+ T cell. The hB7-H2 molecule can be added to the solution containing the cells, or it can be expressed on the surface of an APC, e.g.,
30 an APC presenting an alloantigen or an antigen peptide bound to an MHC molecule.

Alternatively, if the activation is *in vitro*, the hB7-H2 molecule can be bound to the floor or walls of a the relevant culture vessel, e.g., a well of a plastic microtiter plate.

The methods can be performed *in vitro*, *in vivo*, or *ex vivo*. *In vitro* application of hB7-H2 can be useful, for example, in basic scientific studies of immune mechanisms or for production of activated T cells for use in either studies on T cell function or, for example, passive immunotherapy. Furthermore, hB7-H2 could be added to *in vitro* assays (e.g., in T cell proliferation assays) designed to test for immunity to an antigen of interest in a subject from which the T cells were obtained. Addition of hB7-H2 to such assays would be expected to result in a more potent, and therefore more readily detectable, *in vitro* response. However, the methods of the invention will preferably be *in vivo* or *ex vivo* (see below).

The hB7-H2 proteins and variants thereof are generally useful as immune response-stimulating therapeutics. For example, the polypeptides of the invention can be used for treatment of disease conditions characterized by immunosuppression: e.g., cancer, AIDS or AIDS-related complex, other virally or environmentally-induced conditions, and certain congenital immune deficiencies. The compounds may also be employed to increase immune function that has been impaired by the use of radiotherapy or immunosuppressive drugs such as certain chemotherapeutic agents, and therefore are particularly useful when given in conjunction with such drugs or radiotherapy. In addition, in view of the ability of hB7-H2 to co-stimulate the production of especially high levels of IL-10, hB7-H2 molecules can be used to treat conditions involving cellular immune responses, e.g., inflammatory conditions (such as, for example, those induced by infectious agents including *Mycobacterium tuberculosis* or *M. leprae*), or other pathologic cell-mediated responses such as those involved in autoimmune diseases (e.g., rheumatoid arthritis (RA), multiple sclerosis (MS), or insulin-dependent diabetes mellitus (IDDM)).

These methods of the invention can be applied to a wide range of species, e.g., humans, non-human primates, horses, cattle, pigs, sheep, goats, dogs, cats, rabbits, quinea pigs, hamsters, rats, and mice.

In Vivo Approaches

In one *in vivo* approach, the hB7-H2 polypeptide (or a functional fragment thereof) itself is administered to the subject. Generally, the compounds of the invention will be suspended in a pharmaceutically-acceptable carrier (e.g., physiological saline) and administered orally or by intravenous infusion, or injected subcutaneously, intramuscularly, intraperitoneally, intrarectally, intravaginally, intranasally, intragastrically, intratracheally, or intrapulmonarily. They are preferably delivered directly to an appropriate lymphoid tissue (e.g. spleen, lymph node, or mucosal-associated lymphoid tissue (MALT)). The dosage required depends on the choice of the route of administration, the nature of the formulation, the nature of the patient's illness, the subject's size, weight, surface area, age, and sex, other drugs being administered, and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100.0 $\mu\text{g/kg}$. Wide variations in the needed dosage are to be expected in view of the variety of polypeptides and fragments available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization as is well understood in the art. Administrations can be single or multiple (e.g., 2- or 3-, 4-, 6-, 8-, 10-, 20-, 50-, 100-, 150-, or more fold). Encapsulation of the polypeptide in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery, particularly for oral delivery.

Alternatively, a polynucleotide containing a nucleic acid sequence encoding the hB7-H2 polypeptide or functional fragment can be delivered to an appropriate cell of the animal. Expression of the coding sequence will preferably be directed to lymphoid tissue of the subject by, for example, delivery of the polynucleotide to the lymphoid tissue. This can be achieved by, for example, the use of a polymeric, biodegradable microparticle or microcapsule delivery vehicle, sized to optimize phagocytosis by phagocytic cells such as macrophages. For example, PLGA (poly-lacto-co-glycolide) microparticles approximately 1-10 μm in diameter can be used. The polynucleotide is encapsulated in these microparticles, which are taken up by

macrophages and gradually biodegraded within the cell, thereby releasing the polynucleotide. Once released, the DNA is expressed within the cell. A second type of microparticle is intended not to be taken up directly by cells, but rather to serve primarily as a slow-release reservoir of nucleic acid that is taken up by cells only upon release from the micro-particle through biodegradation. These polymeric particles should therefore be large enough to preclude phagocytosis, *i.e.*, larger than 5µm and preferably larger than 20µm.

Another way to achieve uptake of the nucleic acid is using liposomes prepared by standard methods. The vectors can be incorporated alone into these delivery vehicles or co-incorporated with tissue-specific antibodies. Alternatively, one can prepare a molecular conjugate composed of a plasmid or other vector attached to poly-L-lysine by electrostatic or covalent forces. Poly-L-lysine binds to a ligand that can bind to a receptor on target cells [Cristiano et al. (1995), *J. Mol. Med.* 73, 479]. Alternatively, lymphoid tissue specific targeting can be achieved by the use of lymphoid tissue-specific transcriptional regulatory elements (TRE) such as a B lymphocyte, T lymphocyte, or dendritic cell specific TRE. Lymphoid tissue specific TRE are known [Thompson et al. (1992), *Mol. Cell. Biol.* 12, 1043-1053; Todd et al. (1993), *J. Exp. Med.* 177, 1663-1674; Penix et al. (1993), *J. Exp. Med.* 178, 1483-1496]. Delivery of "naked DNA" (*i.e.*, without a delivery vehicle) to an intramuscular, intradermal, or subcutaneous site, is another means to achieve *in vivo* expression.

In the relevant polynucleotides (e.g., expression vectors) the nucleic acid sequence encoding the hB7-H2 polypeptide or functional fragment of interest with an initiator methionine and optionally a targeting sequence is operably linked to a promoter or enhancer-promoter combination.

Short amino acid sequences can act as signals to direct proteins to specific intracellular compartments. For example, hydrophobic signal peptides (e.g., MAISGVPVLGFFIIA VLMSAQESWA (SEQ ID NO:4)) are found at the amino terminus of proteins destined for the ER. While the sequence KFERQ (SEQ ID NO:5) (and other closely related sequences) is known to target intracellular polypeptides to lysosomes, other sequences (e.g., MDDQRDLISNNEQLP (SEQ ID

NO:6) direct polypeptides to endosomes. In addition, the peptide sequence KDEL (SEQ ID NO:7) has been shown to act as a retention signal for the ER. Each of these signal peptides, or a combination thereof, can be used to traffic the hB7-H2 polypeptides or functional fragments of the invention as desired. DNAs encoding the hB7-H2 polypeptides or functional fragments containing targeting signals will be generated by PCR or other standard genetic engineering or synthetic techniques.

A promoter is a TRE composed of a region of a DNA molecule, typically within 100 basepairs upstream of the point at which transcription starts. Enhancers provide expression specificity in terms of time, location, and level. Unlike a promoter, an enhancer can function when located at variable distances from the transcription site, provided a promoter is present. An enhancer can also be located downstream of the transcription initiation site. To bring a coding sequence under the control of a promoter, it is necessary to position the translation initiation site of the translational reading frame of the peptide or polypeptide between one and about fifty nucleotides downstream (3') of the promoter. The coding sequence of the expression vector is operatively linked to a transcription terminating region.

Suitable expression vectors include plasmids and viral vectors such as herpes viruses, retroviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, adenoviruses and adeno-associated viruses, among others.

Polynucleotides can be administered in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to a human, e.g., physiological saline. A therapeutically effective amount is an amount of the polynucleotide which is capable of producing a medically desirable result (e.g., an enhanced T cell response) in a treated animal. As is well known in the medical arts, the dosage for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for administration of polynucleotide is from approximately 10^6 to 10^{12} copies of the polynucleotide molecule. This dose can be repeatedly administered, as needed. Routes of administration can be any of those listed above.

Ex Vivo Approaches

Peripheral blood mononuclear cells (PBMC) can be withdrawn from the patient or a suitable donor and exposed *ex vivo* to an activating stimulus (see above) and a hB7-H2 polypeptide or polypeptide fragment (whether in soluble form or attached to a solid support by standard methodologies). The PBMC containing highly activated T cells are then introduced into the same or a different patient.

An alternative *ex vivo* strategy can involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding an hB7-H2 polypeptide or functional fragment-encoding nucleic acid sequences described above. The transfected or transduced cells are then returned to the subject. While such cells would preferably be hemopoietic cells (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, or B cells) they could also be any of a wide range of types including, without limitation, fibroblasts, epithelial cells, endothelial cells, keratinocytes, or muscle cells in which they act as a source of the hB7-H2 polypeptide or functional fragment for as long as they survive in the subject. The use of hemopoietic cells, that include the above APC, would be particularly advantageous in that such cells would be expected to home to, among others, lymphoid tissue (e.g., lymph nodes or spleen) and thus the hB7-H2 polypeptide or functional fragment would be produced in high concentration at the site where they exert their effect, *i.e.*, enhancement of an immune response. In addition, if APC are used, the APC expressing the exogenous hB7-H2 molecule can be the same APC that presents an alloantigen or antigenic peptide to the relevant T cell. The hB7-H2 can be secreted by the APC or expressed on its surface. Prior to returning the recombinant APC to the patient, they can optionally be exposed to sources of antigens or antigenic peptides of interest, e.g., those of tumors, infectious microorganisms, or autoantigens. The same genetic constructs and trafficking sequences described for the *in vivo* approach can be used for this *ex vivo* strategy. Furthermore, tumor cells, preferably obtained from a patient, can be transfected or transformed by a vector encoding a hB7-H2 polypeptide or functional fragment thereof. The tumor cells, preferably treated with an agent (e.g., ionizing irradiation) that ablates their proliferative capacity, are then returned to the

patient where, due to their expression of the exogenous hB7-H2 (on their cell surface or by secretion), they can stimulate enhanced tumoricidal T cell immune responses. It is understood that the tumor cells which, after transfection or transformation, are injected into the patient, can also have been originally obtained from an individual other than the patient.

The *ex vivo* methods include the steps of harvesting cells from a subject, culturing the cells, transducing them with an expression vector, and maintaining the cells under conditions suitable for expression of the hB7-H2 polypeptide or functional fragment. These methods are known in the art of molecular biology. The transduction step is accomplished by any standard means used for *ex vivo* gene therapy, including calcium phosphate, lipofection, electroporation, viral infection, and biolistic gene transfer. Alternatively, liposomes or polymeric microparticles can be used. Cells that have been successfully transduced are then selected, for example, for expression of the coding sequence or of a drug resistance gene. The cells may then be lethally irradiated (if desired) and injected or implanted into the patient.

Methods of Screening for Compounds that Inhibit or Enhance Immune Response.

The invention provides methods for testing compounds (small molecules or macromolecules) that inhibit or enhance an immune response. Such a method can involve, e.g., culturing a hB7-H2 polypeptide of the invention (or a functional fragment thereof) with T cells in the presence of a T cell activating stimulus (see definition of "activating stimulus" above). The hB7-H2 molecule can be in solution or membrane bound (e.g., expressed on the surface of the APC cells) and it can be natural or recombinant. Compounds that inhibit the T cell response will likely be compounds that inhibit an immune response while those that enhance the T cell response will likely be compounds that enhance an immune response.

A candidate compound whose presence requires at least 1.5-fold (e.g., 2-fold, 4-fold, 6-fold, 10-fold, 150-fold, 1000-fold, 10,000-fold, or 100,000-fold) more hB7-H2 in order to achieve a defined arbitrary level of T cell activation than in the absence of the compound can be useful for inhibiting an immune response. On the other hand,

a candidate compound whose presence requires at least 1.5 fold (e.g., 2-fold, 4-fold, 6-fold, 10-fold, 100-fold, 1000-fold, 10,000 fold, or 100,000-fold) less hB7-H2 to achieve a defined arbitrary level of T cell activation than in the absence of the compound can be useful for enhancing an immune response. Compounds capable of
5 interfering with or modulating hB7-H2 function are good candidates for immunosuppressive immunoregulatory agents, e.g., to modulate an autoimmune response or suppress allogeneic or xenogeneic graft rejection.

The invention also relates to using hB7-H2 or functional fragments thereof to screen for immunomodulatory compounds that can interact with hB7-H2. One of
10 skill in the art would know how to use standard molecular modeling or other techniques to identify small molecules that would bind to T cell interactive sites of hB7-H2. One such example is provided in Broughton [(1997) Curr. Opin. Chem. Biol. 1, 392-398].

15 hB7-H2 Antibodies

The invention features antibodies that bind to the hB7-H2 polypeptide or fragments of the polypeptide. Such antibodies can be polyclonal antibodies present in the serum or plasma of animals (e.g., mice, rabbits, rats, guinea pigs, sheep, horses, goats, cows, or pigs) which have been immunized with the hB7-H2 polypeptide or a
20 peptide fragment of hB7-H2 using methods, and optionally adjuvants, known in the art. Such polyclonal antibodies can be isolated from serum or plasma by methods known in the art.

Monoclonal antibodies that bind to the above polypeptides or fragments are also embodied by the invention. Methods of making and screening monoclonal
25 antibodies are well known in the art. Once the desired antibody-producing hybridoma has been selected and cloned, the resultant antibody can be produced by a number of methods known in the art. For example, the hybridoma can be cultured *in vitro* in a suitable medium for a suitable length of time, followed by the recovery of the desired antibody from the supernatant. The length of time and medium are known or can be
30 readily determined.

Additionally, recombinant antibodies specific for hB7-H2, such as chimeric and humanized monoclonal antibodies comprising both human and non-human portions, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example, using methods described in Robinson et al., International Patent Publication PCT/US86/02269; Akira et al., European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison et al., European Patent Application 173,494; Neuberger et al., PCT Application WO 86/01533; Cabilly et al., U.S. Patent No. 4,816,567; Cabilly et al., European Patent Application 125,023; Better et al. (1988) *Science* 240, 1041-43; Liu et al. (1987) *J. Immunol.* 139, 3521-26; Sun et al. (1987) *PNAS* 84, 214-18; Nishimura et al. (1987) *Canc. Res.* 47, 999-1005; Wood et al. (1985) *Nature* 314, 446-49; Shaw et al. (1988) *J. Natl. Cancer Inst.* 80, 1553-59; Morrison, (1985) *Science* 229, 1202-07; Oi et al. (1986) *BioTechniques* 4, 214; Winter, U.S. Patent No. 5,225,539; Jones et al. (1986) *Nature* 321, 552-25; Veroeayan et al. (1988) *Science* 239, 1534; and Beidler et al. (1988) *J. Immunol.* 141, 4053-60.

Also included within the scope of the invention are antibody fragments and derivatives which contain at least the functional portion of the antigen binding domain of an antibody that binds specifically to hB7-H2. Antibody fragments that contain the binding domain of the molecule can be generated by known techniques. For example, such fragments include, but are not limited to: F(ab')₂ fragments which can be produced by pepsin digestion of antibody molecules; Fab fragments which can be generated by reducing the disulfide bridges of F(ab')₂ fragments; and Fab fragments which can be generated by treating antibody molecules with papain and a reducing agent. See, e.g., National Institutes of Health, 1 Current Protocols In Immunology, Coligan et al., ed. 2.8, 2.10 (Wiley Interscience, 1991). Antibody fragments also include Fv (e.g., single chain Fv (scFv)) fragments, i.e., antibody products in which there are no constant region amino acid residues. Such fragments can be produced, for example, as described in U.S. Patent No. 4,642,334 which is incorporated herein by reference in its entirety.

The following examples are meant to illustrate, not limit, the invention.

Example 1. Materials and Methods

Cells Human embryonic kidney 293 and Chinese hamster ovary (CHO) cells were maintained in DMEM (Gibco BRL, Gaithersburg, MD) containing 10% heat-inactivated fetal bovine serum (FBS) (HyClone, Logan, UT), 25 mM HEPES, 100U/ml penicillin G and 100 mg/ml streptomycin sulfate. Dendritic cells (DC) were generated from adherent PBMC by culturing in RPMI 1640 tissue culture medium supplemented with 10% FBS in the presence of 80 U/ml GM-CSF (Immunex, Seattle, WA) and 500 U/ml IL-4 (R & D, Minneapolis, MN) for 7 days as described [Romani et al., (1994) *J. Exp. Med.* 180, 83-93]. The majority of cells in the culture at day 7 had DC-like morphology with veils and dendritic processes. Fluorescence flow cytometry analysis demonstrated that 99% of these DC-like cells express HLA-DR and >65% of them expresses B7-1. Less than 5% of the cells stained positive with antibodies specific for CD14, CD16, and CD19. Approximately 50% of the DC-like cells in the culture express CD4, but not the T cell markers CD3 and CD8.

Cloning of hB7-H2 cDNA and construction of hB7-H2Ig fusion proteins The NCBI database was screened for amino acid homology to the sequences of human B7-1, B7-2, and B7-H1 using the BLASTN algorithms. The cDNA sequence KIAA0653 in the database was selected for further study. A cDNA fragment containing the hB7-H2 coding sequence was generated by PCR from a human DC cDNA library prepared by SMART PCR cDNA synthesis kit (Clontech, Palo Alto, CA). The sequence of the forward primer for the PCR was 5'-CCGCGGCCCAAGTTCT-3' (SEQ ID NO:8) and the sequence of the reverse primer was 5'-GCCTCATTCCAGGATCACAG-3' (SEQ ID NO:9). Both primers were derived from the KIAA0653 sequence. The resulting PCR product was sequenced using an ABI Prism 310 Genetic Analyzer. The hB7-H2 encoding cDNA was cloned into pcDNA3.1 vector (Invitrogen, Carlsbad, CA) as an EcoRI-HindIII fragment.

hB7-H2Ig fusion genes were created by fusing cDNA encoding the extracellular domain of hB7-H2 in frame with a sequence encoding the CH2-CH3 portion of mouse IgG2a heavy chain (mIg) or human IgG1 heavy chain (hIg) [Dong et al., (1999) *Nature Med.* 5:1365-1369]. The resulting plasmids were transfected into

293 or CHO cells by Fugene 6 (Boehringer-Mannheim) according to manufacturer's instructions. The hB7-H2mIg and hB7-H-2hIg fusion proteins were purified from the culture supernatants of transfected 293 cells grown in serum-free media (GIBCO) by protein G-sepharose column (Pharmacia, Uppsala, Sweden) affinity
5 dichromatography. Human ICOShIg, B7-H1hIg and CTLA4hIg were prepared as described previously [Dong et al., (1999) *Nature Med.* 5:1365-1369].

RNA analysis Northern blot analysis was carried out using Human Multiple Tissue Northern Blots (Clontech, Palo Alto, CA). The membranes were incubated in ExpressHyb hybridization solution (Clontech) for 30 min at 68°C. After labeling by
10 random-priming, a cDNA probe containing the 3' end of hB7-H2 encoding cDNA (750 bp) was heat-denatured and added to fresh hybridization solution. The membrane was hybridized with the solution containing the labeled probe for 16 h at 68°C, washed twice in 2× SSC, 0.05% SDS (30 min at 25°C), once in 0.1× SSC, 0.1% SDS (20 min at 65°C), and exposed at -80°C to x-ray film.

Fluorescence flow cytometry analysis To prepare hB7-H2-specific antisera, BALB/c mice were immunized with purified hB7-H2mIg in complete Freund's adjuvant (CFA) (Sigma) and boosted three times with hB7-H2mIg in incomplete Freund's adjuvant (IFA). Sera were collected 10 days after the last boost. The specificity of the antisera was determined in ELISA against hB7-H2hIg and by
20 fluorescence flow cytometry using 293 cells transfected with a pcDNA3.1 plasmid containing full-length hB7-H2 encoding cDNA (phB7-H2). Preimmunization mouse sera were used in control samples. To detect hB7-H2 expression on DC, 1 × 10⁶ DC were incubated with the above-described antiserum specific for hB7-H2 (1:1000) or control serum (1:1000) in fluorescence flow cytometry buffer (PBS, 3%FCS,
25 0.02%NaN₃) at 4°C for 30 min. The cells were washed and further incubated with FITC-conjugated goat antibody specific for mouse IgG (BioSource, Camarillo, CA) for 30 min at 4°C. Fluorescence was analyzed with a FACSCaliber flow cytometer (Becton Dickinson, Mountain View, CA) with Cell Quest software (Becton Dickinson).

30 To detect the expression of the counter-receptor for hB7-H2, nylon wool-purified T cells were cultured either unstimulated or stimulated with 5 mg/ml PHA

(Sigma) for 1 to 3 days as indicated. The cells were then stained with hB7-H2hIg and analyzed by fluorescence flow cytometry. In addition, 293 cells were transfected by FuGene 6 with the phB7-H2 plasmid. After 48 hours of culture, the transfectants were harvested and stained with antiserum specific for hB7-H2 (1:1000) or ICOShIg. For indirect immunofluorescence staining, cells were further incubated with FITC-conjugated goat antibodies specific for human or mouse IgG, as appropriate. Normal serum or purified human IgG1 was used in control samples.

T cell proliferation and cytokine assays T cell enrichment was performed by passing non-adherent PBMC from healthy human donors through nylon wool columns (Robbins Scientific Co., Sunnyvale, CA) as described previously [Dong et al., (1999) *Nature Med.* 5:1365-1369]. For co-stimulation assays, flat-bottomed 96-well microtiter plates were coated overnight with 100 µl of anti-CD3 mAb (at the indicated concentration) at 4°C. After extensive washing with PBS, the plates were further coated with hB7-H2hIg or control IgG at 37°C for 4 hr. The plates were again extensively washed with PBS and purified T cells were added to the wells at 2 x 10⁵ cells/well. The cells were cultured for 72 hrs and [³H]-thymidine (1.0 mCi/well) was added 15 hrs before harvesting of the cultures. Incorporation of [³H]-thymidine was measured with a MicroBeta Trilux liquid scintillation counter (Wallac, Finland). Each data point is the mean of values obtained from triplicate wells. To detect cytokines, supernatants were collected after 24, 48 or 72 hrs of culture. The concentrations of IL-2 and IL-10 were determined by sandwich ELISA (PharMingen) according to manufacturer's instructions. Culture wells coated with hB7-H1hIg (as described above) and containing mAb specific for human CD28 (PharMingen) in soluble form were included for comparison. Polymyxin B (10 mg/ml) was added to all cultures to completely neutralize any endotoxin contaminating them.

Example 2. Molecular cloning and expression of hB7-H1 encoding cDNA

Searches of the NCBI database using the amino acid sequences of human B7-1, B7-2, and B7-H1 revealed that the N-terminus of the putative protein with accession no. KIAA0653 shared ~20% homology to known B7 members. However, the KIAA0653 putative protein has 558 amino acids and its C-terminus has no

structural similarity to members of the B7 family. Sequencing of a cDNA molecule generated as described above by PCR using primers with sequences derived from the KIAA0653 nucleotide sequence and a human DC cDNA library as a source of template revealed a nucleic acid sequence (SEQ ID NO:3) (FIG. 2A) that differed
5 from that of KIAA0653 downstream of nucleotide 1,027 of KIAA0653. In addition, a stop codon (TGA) 5' of that identified in KIAA0653 was defined. Thus, a new cDNA species (SEQ ID NO:2 (FIG. 2B) consisting of nucleotides 51-956 of SEQ ID NO:3) encoding a human protein with 302 amino acids (designated hB7-H2) was defined by the above strategy.

10 hB7-H2 is a glycosylated, type I membrane protein consisting of a signal peptide, an Ig V-like domain, an Ig C-like domain, a hydrophobic transmembrane domain, and a cytoplasmic tail (FIG. 1A). In addition, hB7-H2 has a conserved tyrosine residue in the Ig V-like domain at position of 80 which is identical to that of B7-1 at position 87, B7-2 at position 82, and hB7-H1 at position 81 (FIG. 1B). Four
15 structural cysteines (labeled by stars in FIG. 1B), which are apparently involved in disulfide bonds forming the Ig V and Ig C domains, are well conserved in all B7 members. hB7-H2 shares overall homology to B7-1 (24% amino acid identity), hB7-2 (21%), and hB7-H1 (21%) based on an analysis using the multiple sequence alignment program, McVector 6.5 software (FIG. 1B).

20 Northern blot analysis revealed that expression of the B7-H2 mRNA is abundant in kidney, PBMC, and brain (FIG. 3). Significant expression was also found in heart, skeletal muscle, spleen and thymus. In most of the tissues that are positive for hB7-H2 mRNA, 3 transcripts of approximately 8, 3.6 and 3 kb were found (FIG. 3), suggesting multiple splice mRNA species for the hB7-H2 gene.
25 RNA-specific PCR analysis of several cDNA libraries showed that hB7-H2 mRNA is detectable in human DC and in Raji and K562 lymphoma cells but not in THP1, Jurkat, and HL60 lymphoma cells.

To determine whether hB7-H2 is expressed as a membrane-bound surface protein, a plasmid containing the cDNA encoding the extracellular region of hB7-H2
30 fused in frame with the CH2-CH3 portion of human IgG1 heavy chain was constructed. The resulting plasmid was transfected into CHO cells and the encoded

fusion protein (hB7-H2hIg) was purified by protein G column from the supernatants of the transfected CHO cells. Antisera against hB7-H2 were prepared by immunization of BALB/c mice with the purified hB7-H2mIg. Fluorescence flow cytometry analysis indicated that, although there is not substantial expression on unfractionated PBMC, DC generated from adherent PBMC in the presence of GM-CSF and IL-4 express hB7-H2 on their surface (FIG. 4). Maturation of DC by further treatment with LPS down-regulated the expression of hB7-H2 although it increased the level of B7-2 expression (FIG. 4). These results indicated preferential expression of hB7-H2 on immature DC.

10

Example 3. Binding of hB7-H2 to the ICOS molecule on activated T cells

The expression of the counter-receptor for hB7-H2 was tested for by fluorescence flow cytometry analysis. Indirect immunofluorescence staining using hB7-H2hIg showed that the counter-receptor is not expressed on resting PBMC (FIG. 5A, 0 hr sample). However, T cells stimulated by PHA expressed high levels of counter-receptor of hB7-H2 (FIG. 5A). The expression can be detected after 24 hrs of stimulation, and is sustained for up to at least 72 hrs. Similar results were obtained using T cells activated with a mixture of antibodies specific for CD3 and CD28. Thus, the counter-receptor of hB7-H2 appears to be inducible in T cells.

20 The ability of soluble ICOShIg or CTLA4hIg to block binding of hB7-H2hIg to activated T cells was tested for. As shown in FIG. 5B, binding of hB7-H2hIg to PHA-activated T cells was completely abrogated by the inclusion of ICOShIg in the staining reaction, but not by the inclusion of CTLA4hIg. These findings indicated that the competition by ICOShIg in the assay was specific and that ICOS is a counter-receptor for hB7-H2. To further test this contention, an experiment was performed to test whether transfection of 293 cells with an expression vector containing the full-length hB7-H2 coding sequence (phB7-H2) could confer ICOS binding activity on the 293 cells. 293 cells transfected with phB7-H2 (293/hB7-H2) were stained by ICOShIg (FIG. 5C, bottom histogram, solid line). This staining was specific since 25 293 cells transfected with parental pCDNA3.1 (293/mock) (FIG. 5C, bottom histogram, dotted line) or with pCDNA3-B7-1 vector did not bind ICOShIg.

30

Importantly, antibody (antiserum) specific for hB7-H2 also bound to 293/hB7-H2 but not to 293/mock cells (FIG. 5C, top histogram, solid line and dotted line, respectively). These results indicate that hB7-H2 binds to ICOS on activated T cells and thus that ICOS is the counter-receptor for hB7-H2.

5

Example 4. hB7-H2 co-stimulates T cell proliferation

To test for co-stimulatory activity in hB7-H2, T cells purified from PBMC of healthy human donors were stimulated with plate-bound hB7-H2hIg in the presence of plate-bound mAb specific for CD3. T cell proliferation was determined by
10 incorporation of [3H]-thymidine after three days of culture. hB7-H2hIg, immobilized on plastic plates at a concentration of 5 mg/ml, enhanced T cell proliferation up to 5-fold compared to the control Ig in the presence of mAb specific for CD3 immobilized on the plates at a concentration of 40 ng/ml (FIG. 6A). The co-stimulatory effects of the hB7-H2hIg were dose-dependent (FIG. 6B). In the absence of mAb specific for
15 CD3, hB7-H2hIg at concentrations up to 5 mg/ml had no effect on T cell proliferation (FIG. 6A). These results demonstrate that hB7-H2 engagement co-stimulates T cell proliferative responses.

Example 5. IL-2 and IL-10 secretion by hB7-H2 co-stimulation

20 To test for the ability of hB7-H2 to co-stimulate production of IL-2 and IL-10, the levels of IL-2 and IL-10 in supernatants from cultures of T cells with plate-bound hB7-H2hIg and mAb specific for CD3 bound to plates at a high concentration (500 ng/ml) were determined by sandwich ELISA. Preliminary experiments demonstrated that T cells proliferated vigorously when activated by this amount of mAb specific for
25 CD3 in the absence of co-stimulation. FIG. 7A shows that hB7-H2hIg significantly increased IL-10 secretion at the 48 hr point but does not affect IL-2 production significantly. In contrast, T cells co-stimulated by plate-bound hB7-H2hIg and a suboptimal dose of mAb specific for CD3 (bound to plates at a concentration of 40 ng/ml) produced IL-2 but relatively little IL-10. IL-10 did not increase over 72 hr
30 while IL-2 was elevated at 48 and further elevated at 72 hrs. As expected, both hB7-

H1hIg and anti-CD28 mAb co-stimulated IL-10 production at both concentrations of mAb specific for CD3. These results indicate that the strength of CD3 engagement affects the pattern of cytokine secretion co-stimulated by hB7-H2 engagement. Moreover, the data indicate that in the presence of an activating stimulus equivalent to
5 a physiological density of peptide-MHC complexes on the surface of APC (*i.e.*, a suboptimal concentration of antibody specific for CD3), hB7-H2 co-stimulates the production of IL-2, but little IL-10. However, in the presence of an activating stimulus equivalent to an abnormally high density of peptide-MHC complexes on the surface of APC (*i.e.*, an optimal concentration of antibody specific for CD3), hB7-H2
10 co-stimulates the production of IL-10, but little IL-2.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

What is claimed is:

1. An isolated DNA comprising:
 - (a) a nucleic acid sequence that encodes a hB7-H2 polypeptide
5 with the ability to co-stimulate a T cell, wherein the encoded polypeptide is less than 555 amino acids in length and the nucleic acid sequence hybridizes under highly stringent conditions to the complement of a sequence that encodes a polypeptide with an amino acid sequence with SEQ ID NO:1; or
 - (b) the complement of the nucleic acid sequence.
- 10 2. The polypeptide of claim 1, wherein,
 - if the polypeptide, when its amino acid sequence is aligned with SEQ ID NO:1, includes a first amino acid residue at a position equivalent to position 301 of wild-type polypeptide (SEQ ID NO:1), the first amino acid residue is histidine or a conservative substitution thereof; or
 - 15 if the polypeptide, when its amino acid sequence is aligned with SEQ ID NO:1, includes a second amino acid residue at a position equivalent to position 302 of wild-type polypeptide (SEQ ID NO:1), the second amino acid residue is valine or a conservative substitution thereof.
- 20 3. The DNA of claim 1, wherein the nucleic acid sequence encodes a polypeptide comprising an amino acid sequence with SEQ ID NO:1.
4. The DNA of claim 1, wherein the nucleic acid sequence has a sequence of SEQ ID NO:2.
5. An isolated polypeptide encoded by the DNA of claim 1.
- 25 6. The isolated polypeptide of claim 5, wherein the polypeptide comprises an amino acid sequence of amino acid residue 22 to amino acid residue 302 of SEQ ID NO:1, or said amino acid sequence but differing solely by conservative substitutions.

7. The isolated polypeptide of claim 5, wherein the polypeptide comprises an amino acid sequence of SEQ ID NO:1, or said amino acid sequence but differing solely by conservative substitutions.
8. A vector comprising the DNA of claim 1.
- 5 9. The vector of claim 8, wherein the nucleic acid sequence is operably linked to a regulatory element which allows expression of said nucleic acid sequence in a cell.
10. A cell comprising the vector of claim 8.
11. A method of co-stimulating a T cell, the method comprising contacting
10 the T cell with the polypeptide of claim 5.
12. The method of claim 11, wherein the contacting comprises culturing the polypeptide with the T cell *in vitro*.
13. The method of claim 11, wherein the T cell is in a mammal.
14. The method of claim 13, wherein the contacting comprises
15 administering the polypeptide to the mammal.
15. The method of claim 14, wherein the contacting comprises administering a nucleic acid encoding the polypeptide to the mammal.
16. The method of claim 13, comprising:
- (a) providing a recombinant cell which is the progeny of a cell
20 obtained from the mammal and has been transfected or transformed *ex vivo* with a nucleic acid encoding the polypeptide so that the cell expresses the polypeptide; and
- (b) administering the cell to the mammal.
17. The method of claim 16, wherein the cell is an antigen presenting cell (APC) and the cell expresses the polypeptide on its surface.
- 25 18. The method of claim 17, wherein, prior to the administering, the APC is pulsed with an antigen or an antigenic peptide.
19. The method of claim 13, wherein the mammal is suspected of having an immunodeficiency disease.

20. The method of claim 13, wherein the mammal is suspected of having an inflammatory condition.
21. The method of claim 13, wherein the mammal is suspected of having an autoimmune disease.
- 5 22. A method of identifying a compound that inhibits an immune response, the method comprising:
- (a) providing a test compound;
 - (b) culturing, together, the compound, the polypeptide of claim 5, a T cell, and a T cell activating stimulus; and
 - 10 (c) determining whether the test compound inhibits the response of the T cell to the stimulus, as an indication that the test compound inhibits an immune response.
23. The method of claim 22, wherein the stimulus is an antibody that binds to a T cell receptor or a CD3 polypeptide.
- 15 24. The method of claim 22, wherein the stimulus is an alloantigen or an antigenic peptide bound to a major histocompatibility complex (MHC) molecule on the surface of an antigen presenting cell (APC).
25. The method of claim 24, wherein the APC is transfected or transformed with a nucleic acid encoding the polypeptide and the polypeptide is
20 expressed on the surface of the APC.
26. A method of identifying a compound that enhances an immune response, the method comprising:
- (a) providing a test compound;
 - (b) culturing, together, the compound, the polypeptide of claim 5, a
25 T cell, and a T cell activating stimulus; and
 - (c) determining whether the test compound enhances the response of the T cell to the antigen, as an indication that the test compound enhances an immune response.

27. The method of claim 26, wherein the stimulus is an antibody that binds to a T cell receptor or a CD3 polypeptide.
28. The method of claim 26, wherein the stimulus is an alloantigen or an antigenic peptide bound to a MHC molecule on the surface of an APC.
- 5 29. The method of claim 28, wherein the APC is transfected or transformed with a nucleic acid encoding the polypeptide and the polypeptide is expressed on the surface of the APC.
30. An antibody that binds specifically to the polypeptide of claim 5.
31. The antibody of claim 30, wherein the antibody is a monoclonal
10 antibody.
32. The antibody of claim 30, wherein the antibody binds to the polypeptide with SEQ ID NO:1.
33. A cell comprising the vector of claim 9.
34. A method of producing a polypeptide that co-stimulates a T cell, the
15 method comprising culturing the cell of claim 33 and purifying the polypeptide from the culture.
35. A fusion protein comprising a first domain joined to at least one additional domain, wherein the first domain comprises a polypeptide of claim 5.
36. The fusion protein of claim 35, wherein the at least one additional
20 domain comprises the constant region of an immunoglobulin heavy chain or a fragment thereof.
37. A nucleic acid molecule encoding the fusion protein of claim 36.
38. A vector comprising the nucleic acid molecule of claim 37.
39. The vector of claim 38, wherein the nucleic acid molecule is operably
25 linked to a regulatory element which allows expression of the nucleic acid molecule in a cell.
40. A cell comprising the vector of claim 39.

41. A method of producing a fusion protein, the method comprising culturing the cell of claim 40 and purifying the fusion protein from the culture.

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signal peptide
 1 MRIGSPGLLFLFFSSLRADTQKEVRAMVGSDVELSCACPEGSRFDLNDVYVYWQTSESKTV
 63 VTYHIPQNSSLENVDSRYRNRALMSPAGMLRGDFSLRLFNVTTPQDEQKFHCLVLSQSLGFQE
 125 VLSVEVTLHVAANFSVVPVVSAPHSPSQDELTFCTTSINGYP RPNVYWINKTDNSLLDQALQN
 187 DTVFLNMRGLYDVVSVLRIARTPSVNI GCCIENVLLQQNLTVGSQTGNDIGERDKITENPVS
 249 TGEKNAATWSILAVLCLLVVAVAIGWVCRDRCLQHSYAGAWAVSPETELTGHV
 transmembrane
 IgC-like domain

FIG. 1A

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GAGGTCTCCGCGCCCCGAGGTCTCCGCGGCCCCGAGGTCTCCGCCCCGCAC
CATGCGGCTGGGCAGTCCTGGACTGCTCTTCCTGCTCTTCAGCAGCCTTCG
AGCTGATACTCAGGAGAAGGAAGTCAGAGCGATGGTAGGCAGCGACGTGG
AGCTCAGCTGCGCTTGCCCTGAAGGAAGCCGTTTTGATTAAATGATGTTTA
CGTATATTGGCAAACCAAGTGAGTCGAAAACCGTGGTGACCTACCACATCCC
ACAGAACAGCTCCTTGGAACAGTGACAGCCGCTACCGGAACCGAGCCC
TGATGTCACCGGCCGGCATGCTGCGGGGCGACTTCTCCCTGCGCTTGTTCA
ACGTCACCCCCCAGGACGAGCAGAAGTTTCACTGCCTGGTGTGAGCCAAT
CCCTGGGATTCCAGGAGGTTTTGAGCGTTGAGGTTACACTGCATGTGGCAG
CAAACCTCAGCGTGCCCGTCGTCAGCGCCCCCACAGCCCCTCCCAGGAT
GAGCTCACCTTCACGTGTACATCCATAAACGGCTACCCCAGGCCCAACGTG
TACTGGATCAATAAGACGGACAACAGCCTGCTGGACCAGGCTCTGCAGAAT
GACACCGTCTTCTTGAACATGCGGGGCTTGATGACGTGGTCAGCGTGCTG
AGGATCGCACGGACCCCCAGCGTGAACATTGGCTGCTGCATAGAGAACGT
GCTTCTGCAGCAGAACCTGACTGTCGGCAGCCAGACAGGAAATGACATCG
GAGAGAGAGACAAGATCACAGAGAATCCAGTCAGTACCGGCGAGAAAAAC
GCGGCCACGTGGAGCATCCTGGCTGTCCTGTGCCTGCTTGTTGGTCGTGGC
GGTGGCCATAGGCTGGGTGTGCAGGGACCGATGCCTCCAACACAGCTATG
CAGGTGCCTGGGCTGTGAGTCCGGAGACAGAGCTCACTGGCCACGTTTGA
CCGGAGCTCACCGCCCAGAGCGTGACAGGGCTTCCATGAGACGCCACCG
TGAGAGGCCAGGTGGCAGCTTGAGCATGGACTCCCAGACTGCAGGGGAGC
ACTTGGGGCAGCCCCCAGAAGGACCACTGCTGGATCCCAGGGAGAACCTG
CTGGCGTTGGCTGTGATCCTGGAATGAGGCCCTTCAAAGCGTCATCCAC
AEGAAAGGCAAATGTCCCCAAGTGAGTGGGCTCCCCGCTGTCACTGCCAGT
CACCCACAGGAAGGGACTGGTGATGGGCTGTCTCTACCCGGAGCGTGCGG
GATTCAGCACCAGGCTCTTCCAGTACCCCAGACCACTGTGGGTCTTCCC
GTGGGATGCGGGATCCTGAGACCGAAGGGTGTGTTGTTTAAAAAGAAGACT
GGGCGTCCGCTCTTCCAGGACGGCCTCTGTGCTGCTGGGGTCACGCGAGG
CTGTTTGCAGGGGACACGGTCACAGGAGCTCTTCTGCCCT (SEQ ID NO:3)

FIG. 2A

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ATGCGGCTGGGCAGTCCTGGACTGCTCTTCCTGCTCTTCAGCAGCCTTCGA
GCTGATACTCAGGAGAAGGAAGTCAGAGCGATGGTAGGCAGCGACGTGGA
GCTCAGCTGCGCTTGCCCTGAAGGAAGCCGTTTTGATTTAAATGATGTTTAC
GTATATTGGCAAACCAAGTGAGTCGAAAACCGTGGTGACCTACCACATCCCA
CAGAACAGCTCCTTGGAACAGTGAGACAGCCGCTACCGGAACCGAGCCCT
GATGTCACCGGCCGGCATGCTGCGGGGCGACTTCTCCCTGCGCTTGTTCA
ACGTCACCCCCCAGGACGAGCAGAAAGTTTCACTGCCTGGTGTTGAGCCAAT
CCCTGGGATTCCAGGAGGTTTTGAGCGTTGAGGTTACACTGCATGTGGCAG
CAAACCTTCAGCGTGCCCGTCGTCAGCGCCCCCACAGCCCCTCCAGGAT
GAGCTCACCTTCACGTGTACATCCATAAACGGCTACCCAGGCCCAACGTG
TACTGGATCAATAAGACGGACAACAGCCTGCTGGACCAGGCTCTGCAGAAT
GACACCGTCTTCTTGAACATGCGGGGCTTGATGACGTGGTCAGCGTGCTG
AGGATCGCACGGACCCCCAGCGTGAACATTGGCTGCTGCATAGAGAACGT
GCTTCTGCAGCAGAACCTGACTGTCGGCAGCCAGACAGGAAATGACATCG
GAGAGAGAGACAAGATCACAGAGAATCCAGTCAGTACCGGCGAGAAAAAC
GCGGCCACGTGGAGCATCCTGGCTGTCCTGTGCCTGCTTGTTGGTCGTGGC
GGTGGCCATAGGCTGGGTGTGCAGGGACCGATGCCTCCAACACAGCTATG
CAGGTGCCTGGGCTGTGAGTCCGGAGACAGAGCTCACTGGCCACGTT
(SEQ ID NO:2)

FIG. 2B

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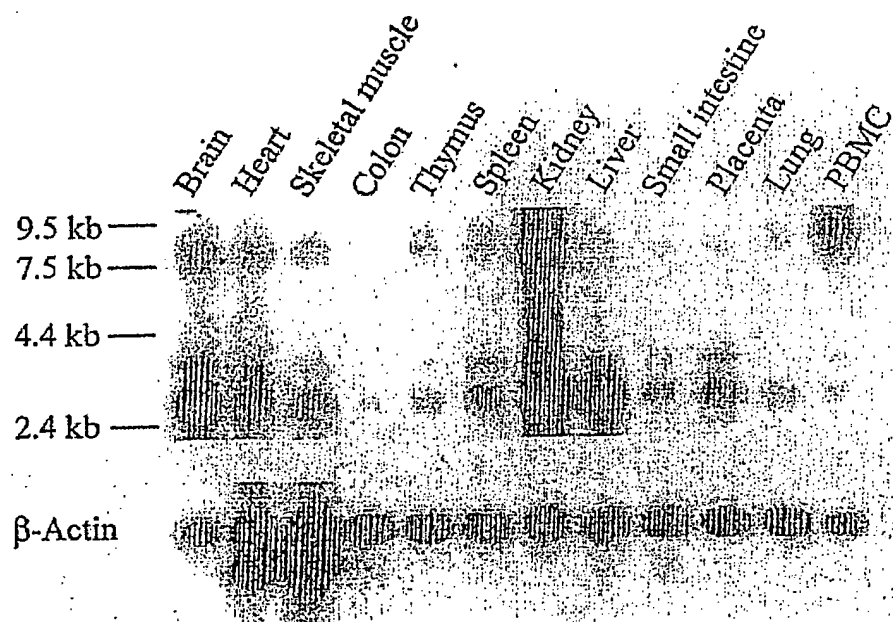


FIG. 3

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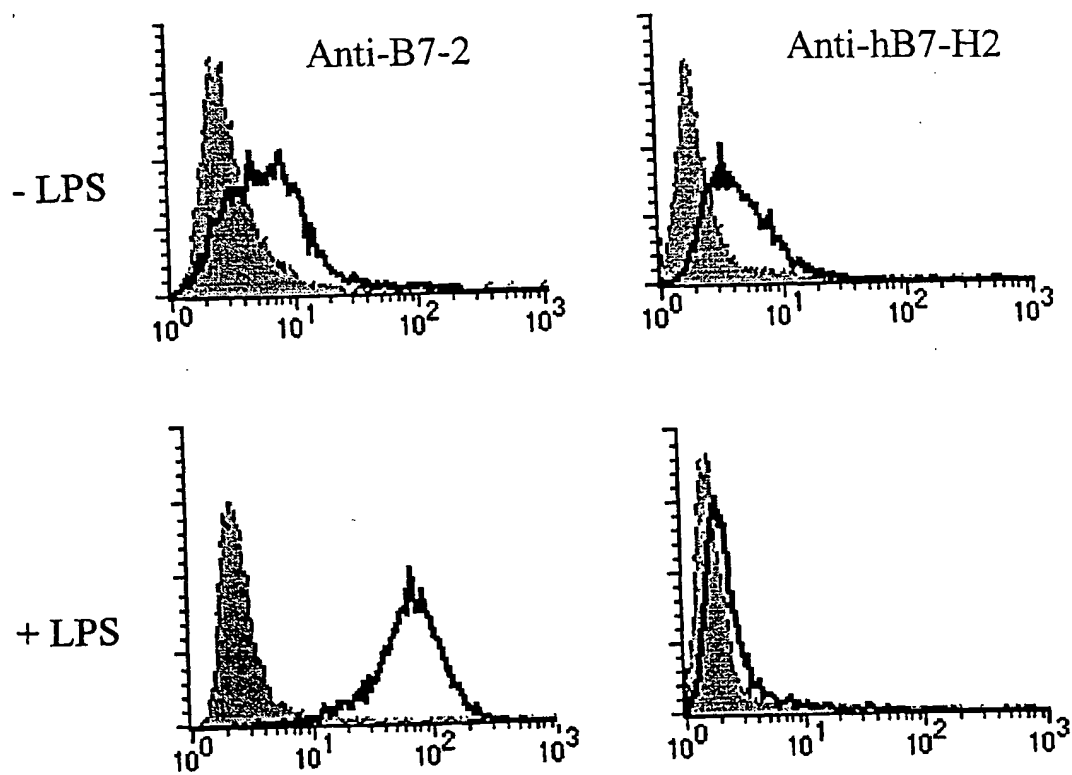


FIG. 4

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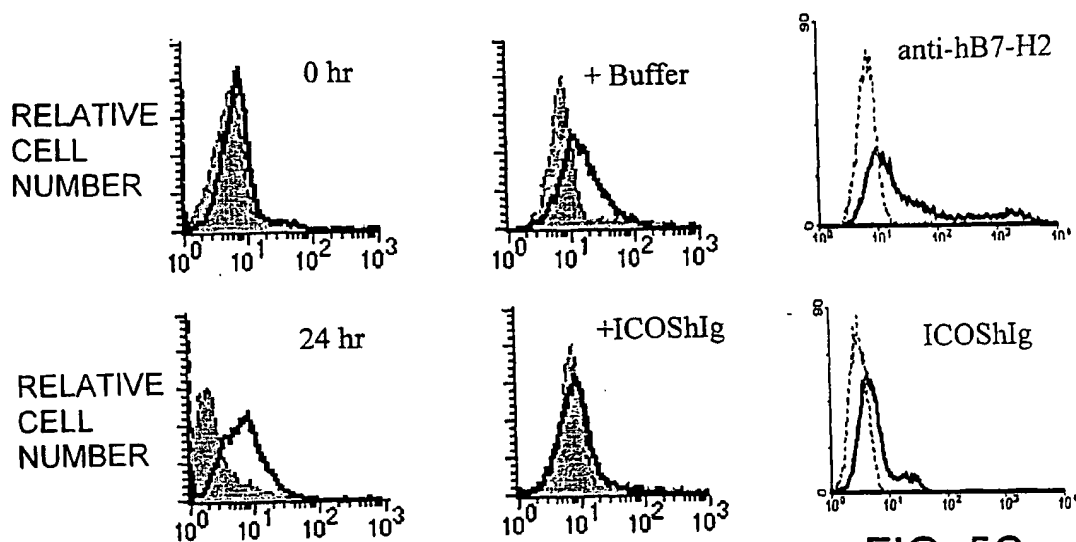


FIG. 5C

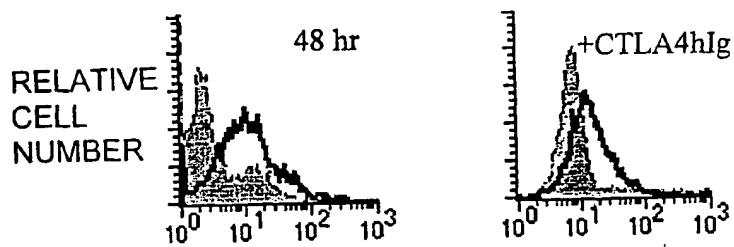


FIG. 5B

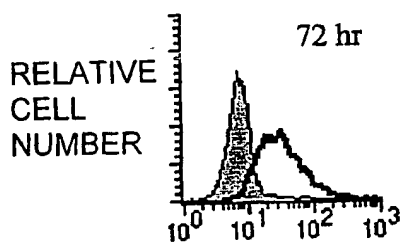


FIG. 5A

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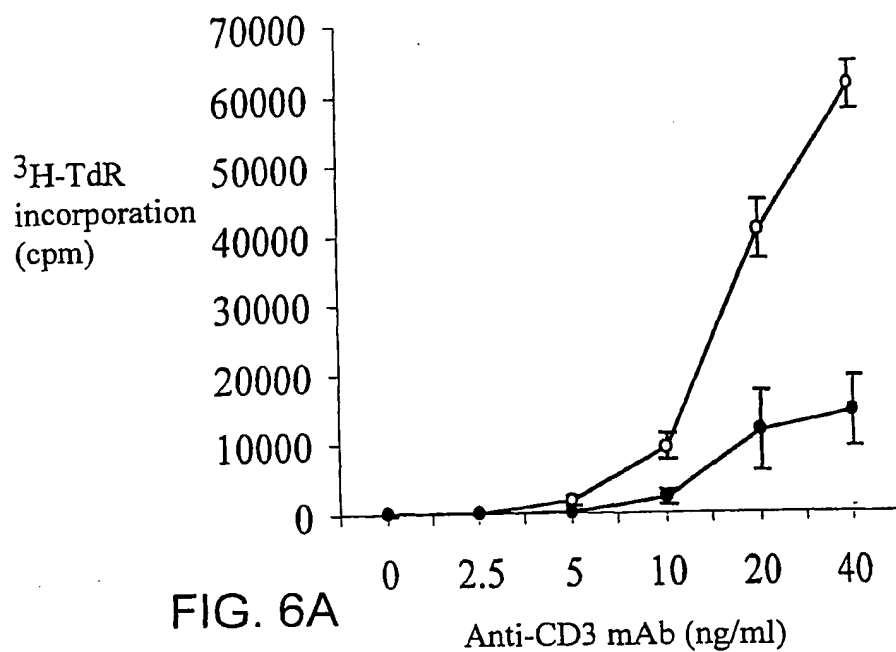


FIG. 6A

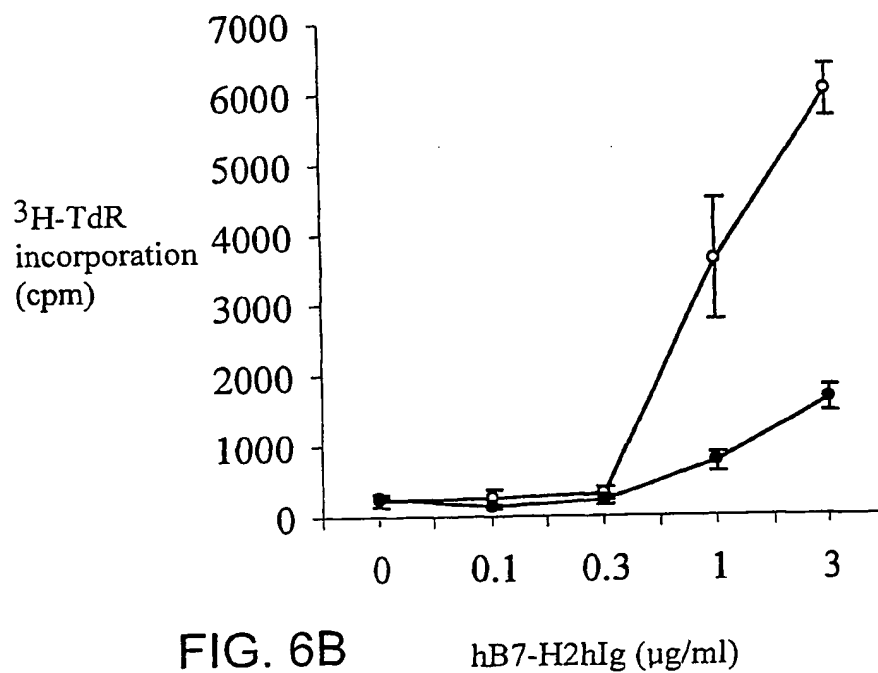


FIG. 6B

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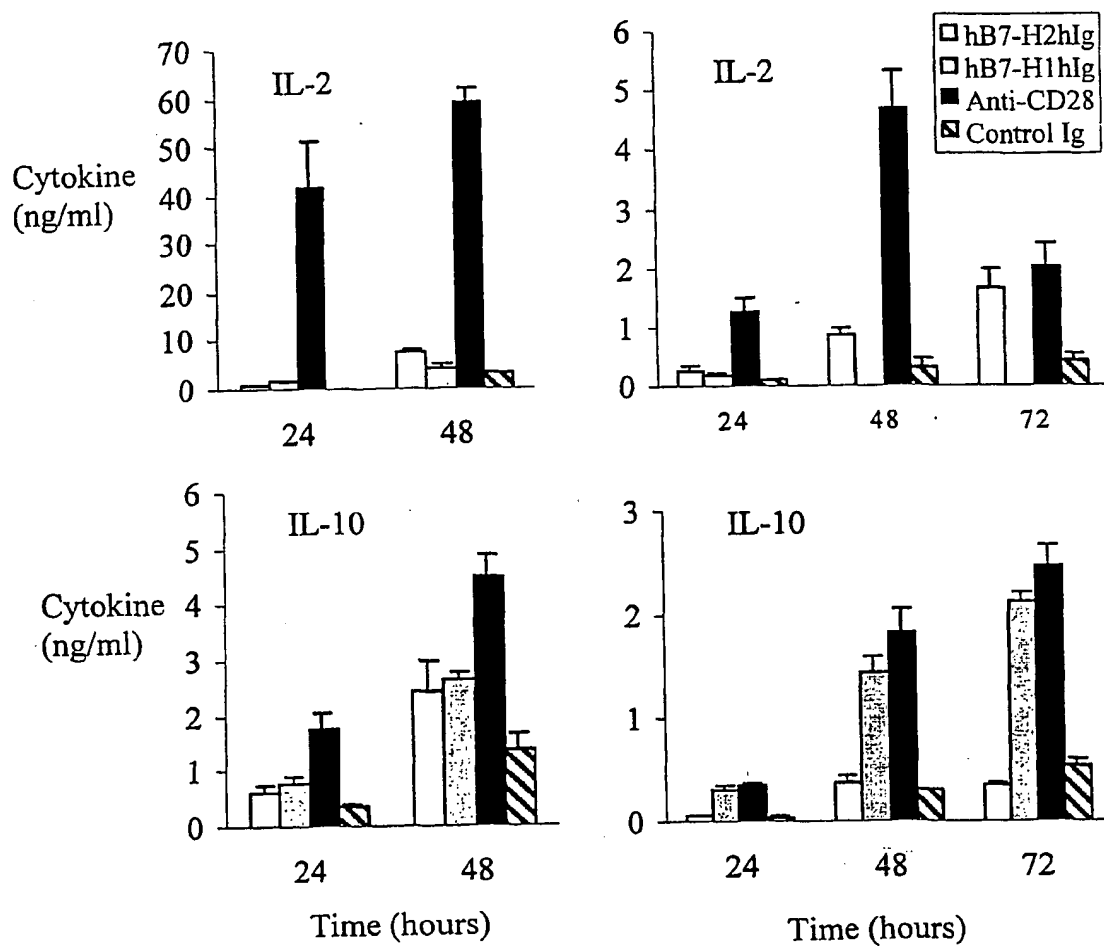


FIG. 7A

FIG. 7B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/06769

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 23.4, 23.5; 530/350, 388.22; 435/7.1, 2, 325, 320.1; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST 2.0, MEDLINE, BIOSIS, EMBASE, CHEM ABS, search terms: author name, hB7-h2, b7?, icos, sequence listing searched

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| Y | ISHIKAWA et al. Prediction of the Coding Sequences of Unidentified Human Genes. X. The Complete Sequences of 100 New cDNA Clones from Brain which can Code for Large Proteins in vitro. DNA Research. 1998, Vol 5, pages 169-176, see entire document. | 1-10, 30-41 |



Further documents are listed in the continuation of Box C.



See patent family annex.

| | |
|--|--|
| * Special categories of cited documents | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" earlier document published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Z" document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

16 JUNE 2001

Date of mailing of the international search report

27 JUL 2001

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/06769

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C07H 21/02, 21/04; A61K 38/16, 38/17; C07K 14/705, 16/28, G01N 33/53

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

536/23.1, 23.4, 23.5; 530/350, 388.22; 455/7.1, 2, 325, 320.1; 514/2